Kidney International, Vol. 3 (1973), p. 238–250

Lanthanum permeability of the tight junction (zonula occludens) in the renal tubule of the rat

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Lanthanum permeability of the tight junction (zonula occludens) in the renal tubule of the rat. The permeability of the tight junctions (*zonulae occludentes*) in the proximal and distal convoluted tubules and the cortical collecting ducts of the rat were evaluated with a lanthanum tracer. Eight non-expanded and nine volume-expanded rats were studied. Non-expanded animals received 0.02 ml/min of isotonic saline. Volume-expanded animals were infused with isotonic bicarbonate-Ringer's solution at the rate of 0.375 ml/min until 10% of the body weight had been administered. Individual proximal and distal tubules from the two groups of animals were preserved for ultrastructural examination by intraluminal microperfusion with glutaraldehyde-formaldehyde or osmium tetroxide followed by microperfusion with lanthanum hydroxide. Some tubules were initially exposed to 1 mM $LaCl_3$ before microperfusion fixation. Additional tubules were preserved via drip-fixation *in situ* and then perfused with lanthanum hydroxide. In volume-expanded animals the pre-expansion values for GFR (1.21 ± 0.08 ml/min), $U_{Na}V$ (0.133 ± 0.028 μEq/min) and EF_{Na} (0.08 ± 0.02%) increased to 1.71 ± 0.17 ml/min, 13.211 ± 1.372 μEq/min, and 6.06 ± 1.16%, respectively. The tight junctions of proximal and distal convoluted tubules were permeable to lanthanum while those of the cortical collecting tubules were impermeable. The results were not detectably different in non-expanded or volume-expanded animals; they were independent of the type and osmolality of the fixative. These results provide morphological evidence for the existence of a paracellular shunt pathway for ion and water movement in the proximal and distal convoluted tubules of the rat.

Les caractéristiques de perméabilité de la jonction serrée (zonula occludens) du tube rénal du rat. Les caractéristiques de perméabilité des jonctions serrées (zonulae occludentes) des tubes contournés proximaux et distaux et des canaux collecteurs corticaux du rat ont été évaluées à l'aide de lanthane. Sept animaux hydropéniques et huit rats ayant subi une expansion extracellulaire ont été étudiés. Les animaux hydropéniques ont reçu du soluté salé isotonique à raison de 0.02 ml/min. Les animaux soumis à une expansion ont été perfusés avec une solution de Ringer bicarbonate au débit de 0.375 ml/min jusqu'à ce que 10% du poids corporel ait été administré. Des tubes proximaux et distaux, dans les deux groupes d'animaux, ont été préservés pour l'étude ultrastructurale au moyen d'une perfusion intraluminale de glutaraldéhyde—formaldéhyde ou de tétraoxyde d'osmium suivie d'une microperfusion d'hydroxyde de lanthane. Quelques tubules ont été initialement exposés à 1 mM $LaCl_3$ avant la fixation par microperfusion. D'autres tubules ont été préservés par une fixation goutte à goutte in situ puis perfusés avec de l'hydroxyde de lanthane. Chez les animaux soumis à une expansion les valeurs initiales de GFR (1.21 ± 0.08 ml/min), $U_{Na}V$ (0.133 ± 0.028 μEq/min) et EF_{Na} (0.08 ± 0.02%) ont augmenté jusqu'à 1.71 ± 0.17 ml/min, 13.211 ± 1.372 μEq/min, et 6.06 ± 1.16% respectivement. Les jonctions serrées des tubes contournés proximaux et distaux étaient perméables au lanthane alors que celles des tubes collecteurs corticaux étaient imperméables. Les résultats n'étaient pas différents, dans les limites de la détection, chez les animaux hydropéniques et soumis à une expansion; ils étaient indépendants du type et de l'osmolalité du fixateur. Ces résultats fournissent une preuve morphologique de l'existence d'une voie de shunt paracellulaire pour le mouvement d'eau et d'ions dans les tubes contournés proximaux et distaux.

An increasing body of evidence suggests that epithelia capable of active sodium transport can be divided into two main groups on the basis of certain functional and structural characteristics of their tight junctions, those whose tight junctions are actually "tight" and those whose junctions appear to be relatively "leaky" to the movement of ions and water [1, 2]. This division is based, in part, on measurements of transepithelial electrical resistance which have permitted the separation of epithelia into those whose resistances are relatively low ($<200\ \Omega\text{-cm}^2$) or high ($>1750\ \Omega\text{-cm}^2$). Epithelia of the latter group include the toad urinary bladder ($2400\ \Omega\text{-cm}^2$) [3], the turtle urinary bladder ($1750\ \Omega\text{-cm}^2$) [4] and frog skin ($2500\text{–}5000\ \Omega\text{-cm}^2$) [5]. A partial list of tissues with relatively low transepithelial

Received for publication August 31, 1972;
accepted in revised form November 17, 1972.

electrical resistance includes the rat renal proximal tubule (5 to 7 Ω-cm^2) [6], rat colon (140 Ω-cm^2) [7], rat jejunum (61 Ω-cm^2) [8], rat ileum (60 to 80 Ω-cm^2) [9], rabbit gall bladder (29 Ω-cm^2) [1], rabbit ileum (21 Ω-cm^2) [10, 11], and *Necturus* gall bladder (307 Ω-cm^2) [2]. The low transepithelial electrical resistance of this latter group has been attributed, at least in part, to the presence of a functionally significant low resistance shunt in parallel with the higher resistance across the cell membrane. For example, in the proximal tubule of the rat the intracellular electrical potential has been found to be as high as 80 mv (interior negative) while the transtubular electrical resistance has been recorded at no more than 5 to 7 Ω-cm^2 [6, 12]. These observations are best explained by the movement of ions and water through a low resistance intercellular shunt, and the lateral intercellular space and the tight junction have been thought to provide the anatomic pathway for such a shunt.

Recent morphological observations have supported the presence of functional transepithelial shunts in epithelia with low resistances. Machen, Erlij and Wooding [1] and Whittembury and Rawlins [13] have demonstrated that lanthanum, an electron-dense tracer confined to the extracellular space, is capable of penetrating the tight junction and entering the lateral intercellular space of rabbit gall bladder and ileum and the proximal tubule of toad kidney, respectively. On the other hand, in similar experiments, Martinez-Palomo, Erlij and Bracho [14] were unable to demonstrate lanthanum penetration of the tight junction of frog skin, a high resistance epithelium.

The present study was initiated to provide morphological evidence for the presence or absence of paracellular shunt pathways for ion and water movement in various segments of the rat nephron, including the proximal and distal convoluted tubule and the cortical collecting duct.

Methods

Seventeen female Sprague-Dawley rats weighing 180 to 250 g were used. The animals were fasted overnight but free access to water was permitted. All animals were anesthetized with intraperitoneal sodium pentobarbital (35 mg/kg body wt) before tracheostomy and cannulation of the carotid artery and jugular vein were performed. The animals were placed on a thermostatically controlled warming table and the left kidney was mobilized through an abdominal incision and prepared for micropuncture as previously described [15]. A polyethylene (P. E. 50) catheter was inserted into the urinary bladder via a suprapubic incision. Estimates of surgical blood loss were replaced initially with isotonic saline (approximately 2 ml). Mean arterial blood pressure was monitored throughout the entire procedure.

All animals were treated identically during the first two hours of the experimental period: inulin (100 mg/ml) in 0.85% saline was infused intravenously at a rate of 0.02 ml/min. After the first 60 min period of equilibration, three 20 min urine collections and simultaneous samples of blood (200 µl/sample) were obtained sequentially for the determination of baseline inulin clearance, urinary sodium excretion, and urine flow. When baseline clearance periods had been obtained, the animals were handled in one of two ways.

Non-expanded animals. In five animals the infusion of isotonic saline was continued for the next 60 to 90 min at the baseline rate of 0.02 ml/min; the collection of blood and urine samples for sequential clearance periods was continued as well. During this period, the surface convolutions of four to six proximal or distal tubules were identified, a small-tipped micropipette was inserted into the tubule lumen, and the individual segments were perfused for three to five minutes with either a glutaraldehyde-formaldehyde fixative solution containing 0.5% lissamine green (osmolality: 960 mOsm/kg) or 1% osmium tetroxide in *s*-Collidine buffer (osmolality: 190 mOsm/kg H_2O). After fixation, the perfusion pipette was withdrawn and a second pipette containing a lanthanum solution (prepared according to the method of Revel and Karnovsky [16]) was then introduced at the same or a nearby puncture site and the lumen was perfused with lanthanum for three to five minutes. Visible distention and retrograde filling of the tubule lumen were carefully avoided during microperfusion. In one of the animals lanthanum was intentionally infiltrated into the adjacent interstitium around proximal and distal convolutions that had been previously fixed by intraluminal microperfusion (but not exposed to intraluminal lanthanum) to determine whether the extracellular tracer could enter the tight junction from the peritubule surface. Following microperfusion each kidney was removed and the individual tubules were dissected free of most of the surrounding unfixed tissue. Renal tubules initially fixed with glutaraldehyde-formaldehyde were post-fixed in one per cent osmium tetroxide for one to two hours. Renal tubules initially fixed in osmium tetroxide were fixed for an additional hour in one or two percent osmium tetroxide. All tubules were then dehydrated in a graded series of alcohols and infiltrated and embedded in Epon epoxy resin [17] in a routine manner for light and electron microscopy. Lanthanum was not added to either the osmium tetroxide solution during post-fixation or to the alcohols that were used for dehydration.

To minimize the possibility that the morphological results were influenced by an elevation in intraluminal pressure which might have occurred during the initial phase of microperfusion fixation, the left kidney of an additional non-expanded animal was preserved by drip-fixation using the same glutaraldehyde-formaldehyde solution. The kidney was mobilized and supported in preparation for micropuncture. After appropriate plasma and urine collections were obtained, the surface of the kidney was drip-fixed *in situ* for 30 min [18]. Surface convolutions of proximal and distal tubules were then identified and the lanthanum solution was microperfused into the tubule lumen for three to five minutes. The individually perfused tubules were then

excised, post-fixed in osmium tetroxide and processed in a routine manner for light and electron microscopy.

Acute volume-expanded animals. The influence of acute volume expansion on the appearance of the tight junction was evaluated in eight additional rats. After baseline clearance periods had been obtained, each of these animals received an intravenous infusion of isotonic bicarbonate-Ringer's solution [19] at a rate of 0.375 ml/min until a volume equal to 10% of the body weight of each animal had been given. The infusion rate was then adjusted to approximate the loss of urine during the ensuing 60 to 90 min period. Blood samples and timed urine collections were obtained sequentially. The surface convolutions of individual proximal and distal tubules were identified and microperfused with fixative followed by lanthanum. An additional group of six tubules was fixed by microperfusion but was not exposed to the lanthanum tracer. In two of the animals, lanthanum was again intentionally infiltrated into the adjacent interstitium around proximal and distal convolutions that had been previously fixed by intraluminal microperfusion as described in an earlier section. All subsequent tissue processing was performed in a manner identical to that used for tubules from the non-expanded animals.

In three additional animals (two non-expanded and one expanded) a solution of $LaCl_3$ (1 mM) was introduced first into the tubule lumen to exclude the possibility that fixation with either osmium tetroxide or glutaraldehyde-formaldehyde altered the structural integrity of the tight junctions before the tubules were exposed to lanthanum. In these animals the individual tubules were initially microperfused for three to 20 min with $LaCl_3$ *before* microperfusion fixation. To prepare the perfusing solution, lanthanum (La^{3+}) was added as $LaCl_3$ to isotonic saline in a final concentration of 1 mM. Previous studies in other transporting epithelia have failed to demonstrate any significant functional or structural alteration upon exposure to La^{3+} in this concentration [1]. After exposure to lanthanum, the individual tubules underwent microperfusion fixation; they were then excised, post-fixed in osmium tetroxide and processed for light and electron microscopy in the usual manner. Again, lanthanum was not added to the post-fixation solution or to the alcohols that were used for dehydration.

Sections for light microscopy were cut one micron in thickness on a Porter-Blum ultramicrotome and stained with toluidine blue [20]. Thin sections were cut for electron microscopy and doubly stained with 3% uranyl acetate [21] and lead citrate, stained with uranyl acetate or lead citrate alone, or left unstained before examination in an AEI 6B electron microscope. A total of 42 individual tubules from 17 animals were examined by light and electron microscopy.

Inulin concentrations in plasma and urine were measured by the anthrone method of Führ, Kaczmarczyk and Krüttgen [22]. The concentration of sodium in plasma and urine was determined by standard flame photometry.

Numerical data were analysed by Student's *t*-test. Data are expressed as mean ± standard error and P values greater than 0.05 are considered to be non-significant (*NS*).

Results

Clearance data. The results of sequential clearance observations performed in the non-expanded and volume-expanded animals are shown in Table 1. Inulin clearance, absolute ($U_{Na}V$) and fractional (EF_{Na}) sodium excretion and urine flow were equal in the two groups during the initial periods of baseline observation. As expected these values rose significantly in the volume-expanded rats. The hematocrit was also similar initially in the two groups but fell significantly with volume-expansion. This response to volume-expansion is quite comparable to that obtained by Brenner, Troy and Daugharty [18] using an identical method of expansion.

Table 1. Clearance data

		Non-expanded rats	Expanded rats	P
Hct	B	52.3 ± 1.1	52.3 ± 1.5	*NS*
%	P	50.9 ± 0.6	38.4 ± 1.5	<0.001
BP	B	111 ± 4	107 ± 6	*NS*
mmHg	P	103 ± 2	108 ± 5	*NS*
V	B	5.9 ± 0.9	5.9 ± 0.6	*NS*
μl/min	P	5.8 ± 0.9	64.5 ± 8.9	<0.001
GFR	B	0.93 ± 0.15	1.21 ± 0.08	*NS*
ml/min	P	0.84 ± 0.15	1.71 ± 0.17	<0.01
$U_{Na}V$	B	0.121 ± 0.038	0.133 ± 0.028	*NS*
μEq/min	P	0.203 ± 0.040	13.211 ± 1.372	<0.001
EF_{Na}	B	0.13 ± 0.05	0.08 ± 0.02	*NS*
%	P	0.24 ± 0.08	6.06 ± 1.16	<0.001

Abbreviations: B = baseline period; P = microperfusion period; Hct = hematocrit; BP = blood pressure; V = urine flow rate; GFR = glomerular filtration rate; $U_{Na}V$ = urinary sodium excretion rate; EF_{Na} = excreted fraction of filtered sodium.

Morphological studies. The typical appearance of electron-dense lanthanum deposits along the brush border of a proximal convoluted tubule from a non-expanded animal is demonstrated in Fig. 1. This tubule was perfusion-fixed before exposure to lanthanum. The deposits surround the individual microvilli that form the brush border and extend into the deep invaginations of the apical plasmalemma between the microvilli. The apparent presence of lanthanum within many of the apical vesicles can be explained by the plane of section passing through the deep and tortuous apical invaginations. Deposits of lanthanum were not observed within the cells unless obvious cellular damage, probably mechanical in nature and occurring at the time of the microperfusion fixation, was also present. The tight junction or *zonula occludens* of the proximal convoluted tubule of the rat nephron is quite shallow; it ranges

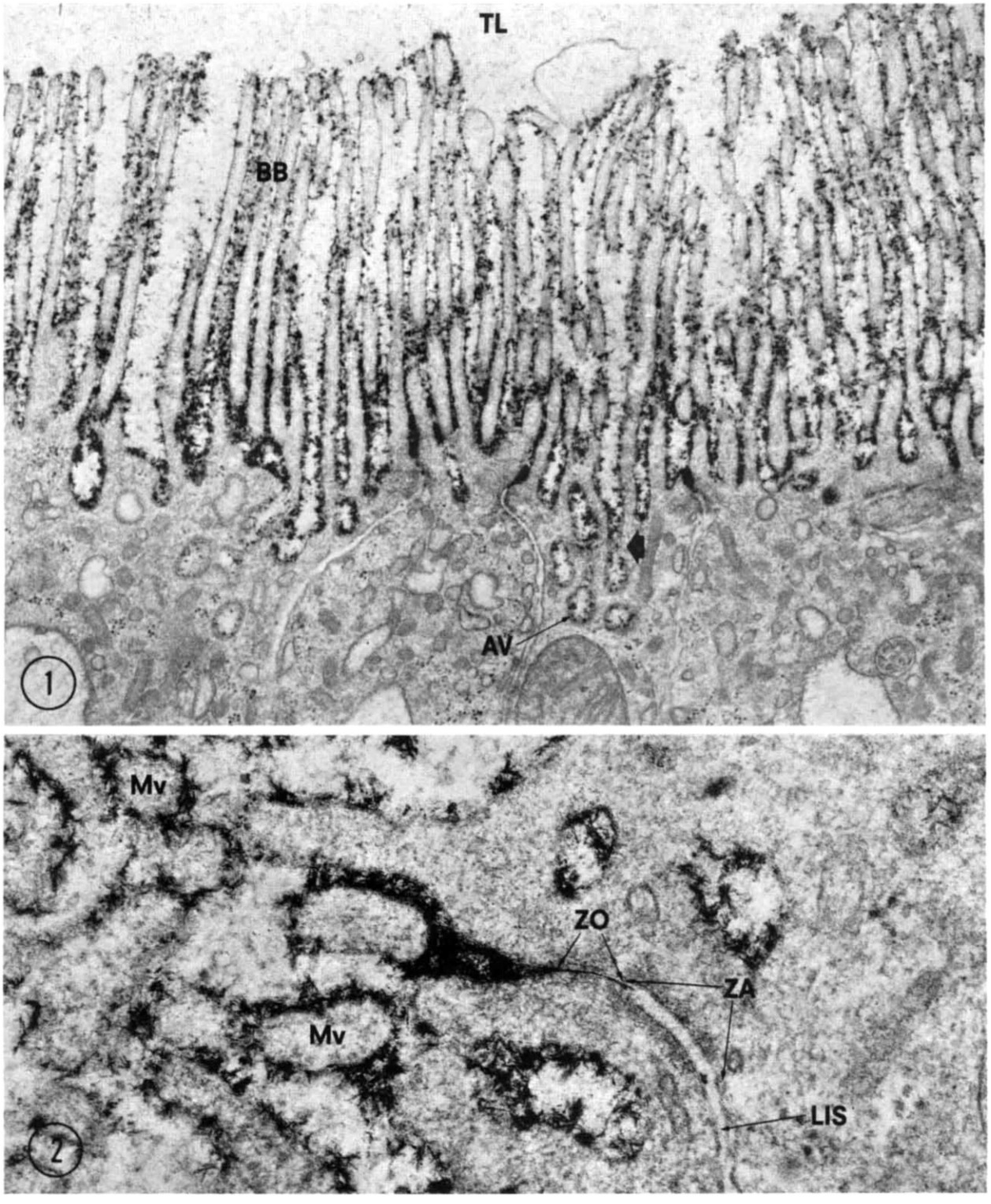

Fig. 1. *Section of apical region in the epithelium of a proximal convoluted tubule from a non-expanded rat.* Electron-dense lanthanum deposits surround the individual microvilli that form the brush border (BB). Lanthanum has entered invaginations of the apical plasmalemma (arrow). The apparent presence of lanthanum within apical vesicles (AV) of the cell is probably due to the plane of section passing through the tortuous apical invaginations. TL, tubule lumen. Primary fixation with glutaraldehyde-formaldehyde. Sections doubly stained with uranyl acetate and lead citrate (× 22,500).

Fig. 2. *Oblique section through apical region of epithelium in a proximal convoluted tubule from a non-expanded rat.* Individual microvilli (Mv) in oblique and cross section on the left are surrounded by lanthanum. Lanthanum has penetrated the entire *zonula occludens* (ZO) from the luminal surface to the region of the *zonula adherens* (ZA). LIS, lateral intercellular space. Primary fixation with glutaraldehyde-formaldehyde. Sections doubly stained with uranyl acetate and lead citrate (× 90,000).

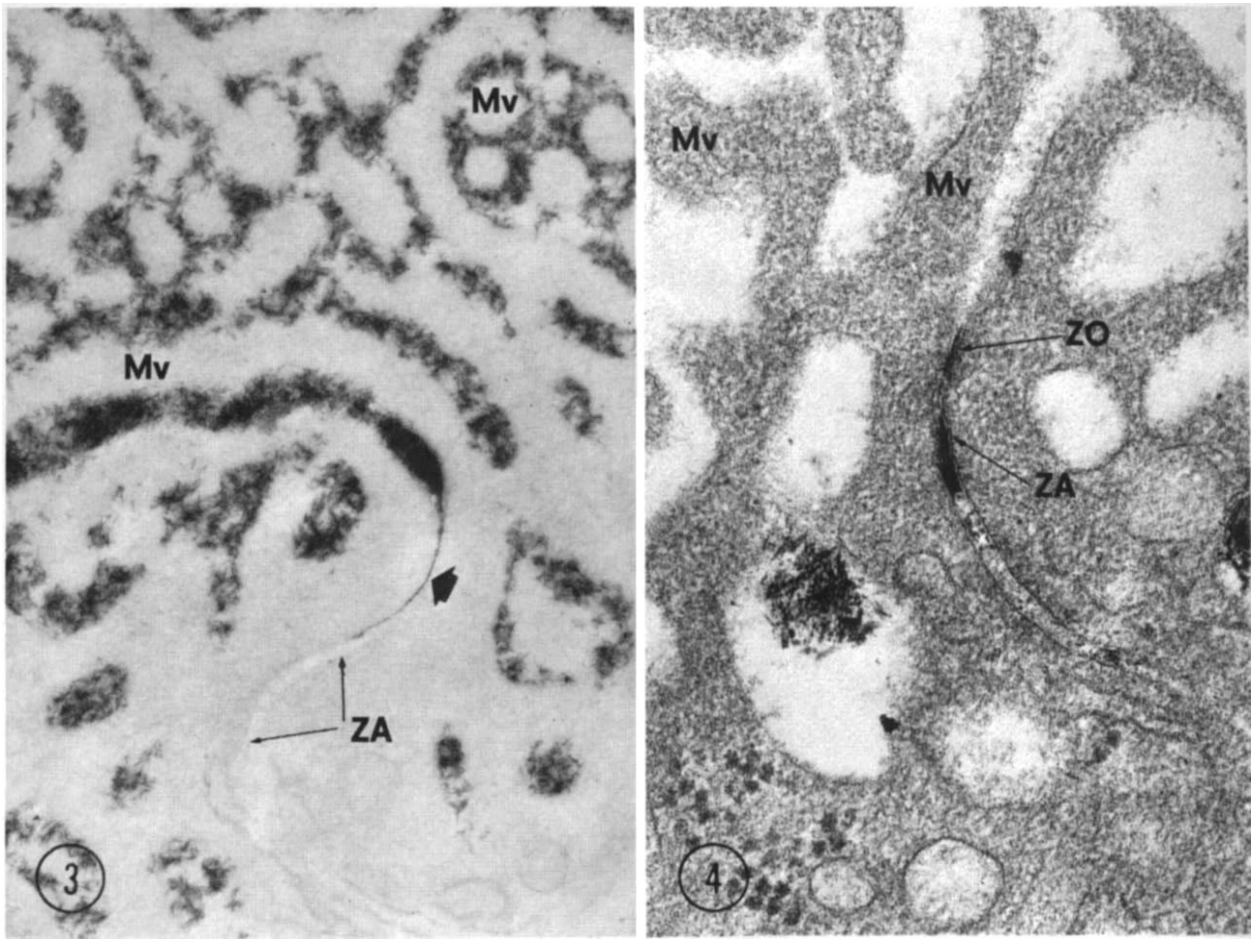

Fig. 3. *Section through the apical region of proximal convoluted tubule epithelium of a non-expanded rat.* The tubule lumen, though not readily apparent, is located at the top of the figure. As demonstrated in Fig. 2, individual microvilli (Mv) are surrounded by lanthanum. Lanthanum has penetrated the entire *zonula occludens* (arrow) which is shown in oblique view. ZA, *zonula adherens*. Primary fixation in glutaraldehyde-formaldehyde. Section is unstained (× 67,000).

Fig. 4. *Section through the apical region of proximal convoluted tubule epithelium of a volume-expanded rat.* Lanthanum has penetrated the *zonula occludens* (ZO) and extends into the region of the *zonula adherens* (ZA). Mv, microvilli. Primary fixation in glutaraldehyde-formaldehyde. Section doubly stained with uranyl acetate and lead citrate (× 99,000).

from 200 Å to 400 Å in depth and forms a belt-like structure around the entire cell [23]. Lanthanum deposits appeared to have penetrated the tight junction between individual cells of the proximal convoluted tubule in both non-expanded and volume-expanded animals (Figs. 2–5). The deposits often extended into the region of the intermediate junction or *zonula adherens* and the lateral intercellular space. However, lanthanum was never deposited extensively within the lateral intercellular space when it seemed to have entered solely via the tight junction. There appeared to be no difference in the frequency or extent of penetration of lanthanum into the tight junctions in the two physiological conditions. Its presence in the tight junctions appeared to correlate best with the total amount of lanthanum present within the tubule lumen.

In the distal convoluted tubule, lanthanum was also observed to penetrate the tight junctions between individual cells. The penetration of lanthanum was still complete even though the depth (0.3 μ) of the tight junction in the distal convoluted tubule of the rat is much greater than that of the proximal tubule [23] (Fig. 6). Differences were not observed between non-expanded and volume-expanded animals.

In striking contrast to observations in the proximal and distal convoluted tubules, the tight junction in the cortical collecting duct resisted lanthanum penetration (Figs. 7–9). Lanthanum did not appear to enter the tight junction from either the antiluminal (Fig. 8) or the luminal surface (Fig. 9). Again, the results were identical in the two physiological conditions.

In the one non-expanded and two volume-expanded animals in which lanthanum was intentionally infiltrated into the interstitium around tubules previously fixed by intraluminal microperfusion, heavy deposits of lanthanum were observed within the basilar and lateral intercellular

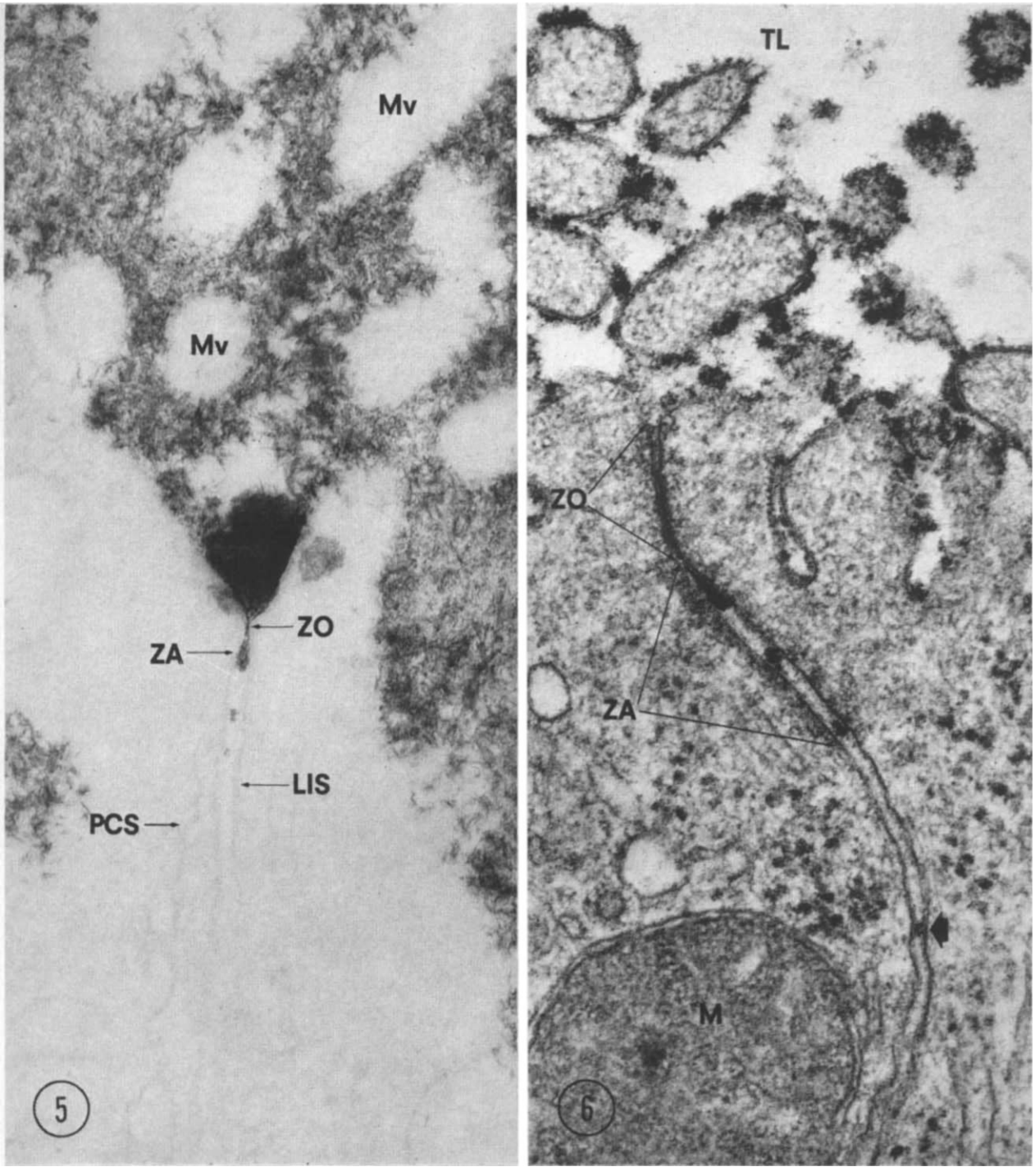

Fig. 5. *Section through the apical region of epithelium of the proximal convoluted tubule of a volume-expanded rat.* Individual microvilli (Mv) are surrounded by lanthanum. Lanthanum has penetrated the entire *zonula occludens* (ZO) and extends into the region of the *zonula adherens* (ZA). LIS, lateral intercellular space; PCS, paramembranous cisternal system. Primary fixation in glutaraldehyde-formaldehyde. Section is unstained (× 100,000).

Fig. 6. *Section through the apical region of epithelium of the distal convoluted tubule of a non-expanded rat.* Lanthanum is present within the *zonula occludens* (ZO) and has entered the region of the *zonula adherens* (ZA) and the lateral intercellular space (arrow). TL, tubule lumen; M, mitochondrion. Tissue preserved by drip-fixation *in situ* with glutaraldehyde-formaldehyde. Section doubly stained with uranyl acetate and lead citrate (× 93,000).

spaces of proximal and distal convoluted tubules and cortical collecting ducts (Figs. 8 and 10). Examination of the junctional complexes revealed that lanthanum had penetrated the intermediate and tight junctions in the proximal and distal convoluted tubules via the retrograde route without difficulty (Fig. 11) but retrograde penetration of the tight junction of the cortical collecting ducts was not observed (Fig. 8).

The results of the experiments in which lanthanum was perfused into tubules that had previously been preserved by drip-fixation rather than by microperfusion fixation are depicted in Figs. 6 and 12. Again, lanthanum deposits were

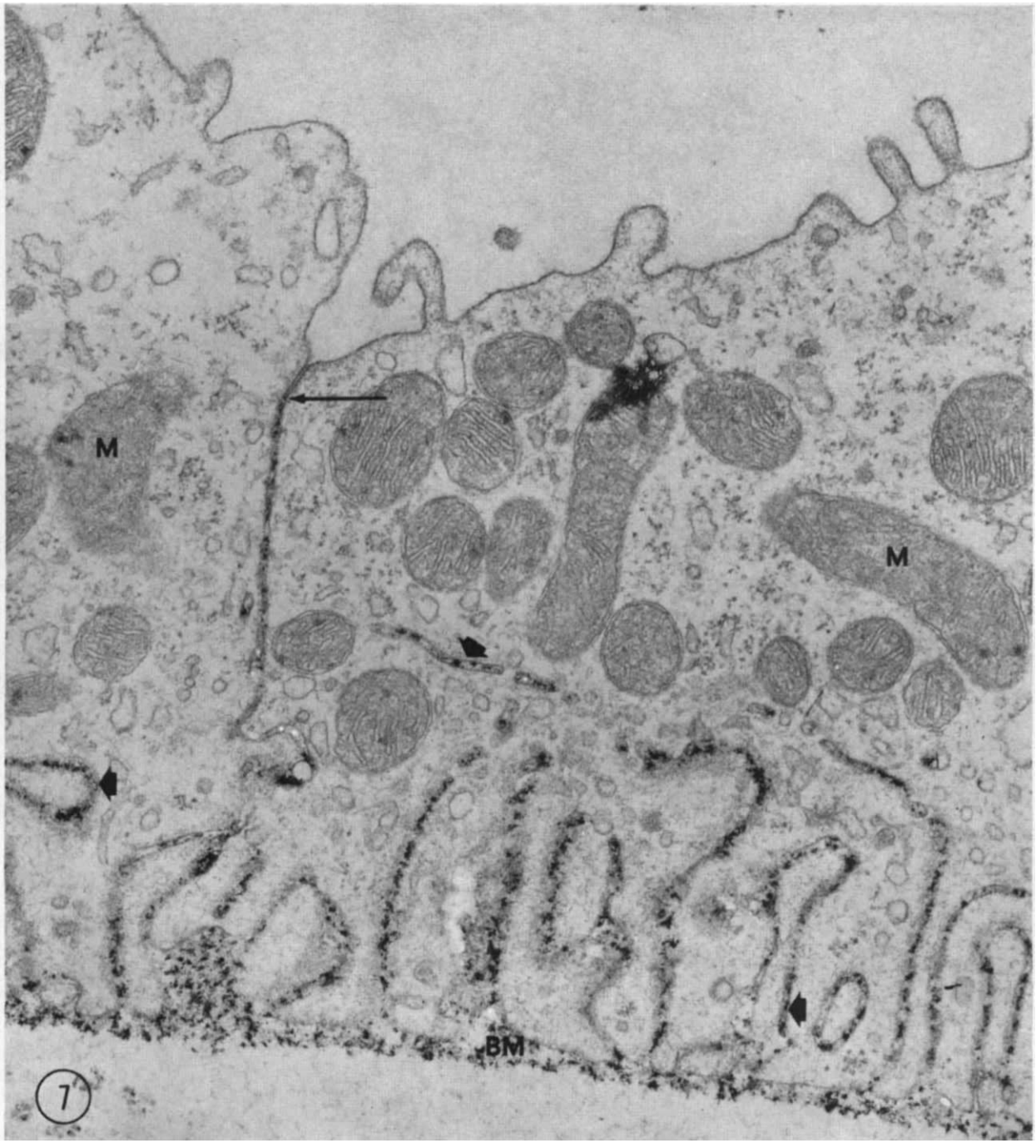

Fig. 7. *Section of cortical collecting duct epithelium demonstrating lanthanum within the lateral and basilar intercellular spaces (large arrows) which entered from the peritubule surface after infiltration directly into the surrounding interstitium.* The tubule lumen is at the top and the interstitium is at the bottom. Note that the column of lanthanum stopped abruptly at the antiluminal surface of the *zonula occludens* (small arrow). BM, basement membrane; M, mitochondrion. Primary fixation in osmium tetroxide. Section is doubly stained with uranyl acetate and lead citrate (× 34,000).

found within the tight junctions of proximal and distal convoluted tubules, thus suggesting that lanthanum penetration of the tight junction was not primarily dependent upon an artifactual elevation in intratubular pressure that might have occurred inadvertently during microperfusion fixation.

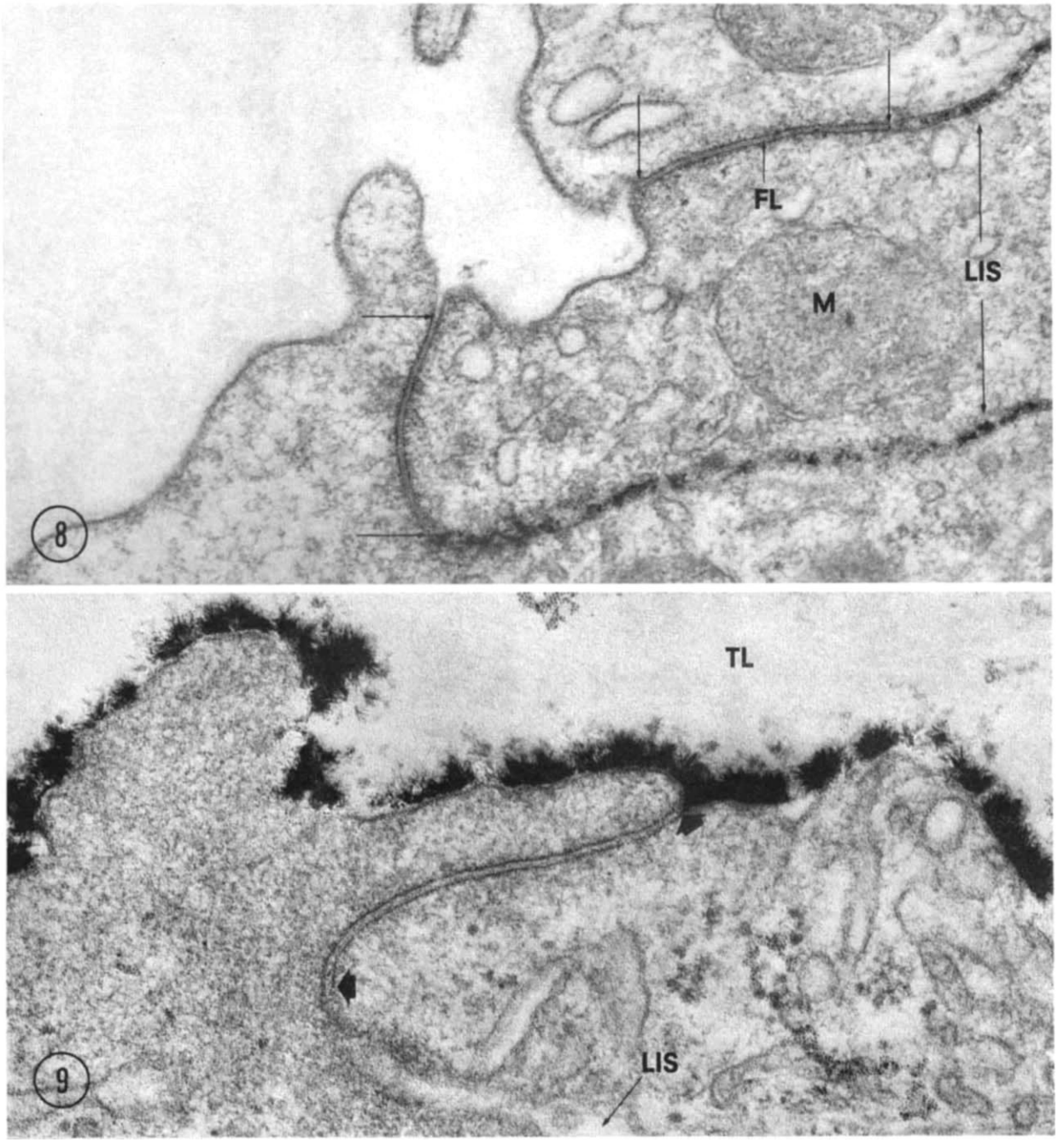

Fig. 8. *Section through apical region of cortical collecting duct in a volume-expanded rat showing two junctional complexes between contiguous cells.* The tubule lumen is at the left. The *zonulae occludentes* extend between the arrows. A thin electron dense line (FL) is evident where the outer leaflets of the two adjacent cell membranes have "fused" giving the characteristic pentalaminar configuration of the *zonula occludens*. Lanthanum has entered the lateral intercellular space (LIS) via a retrograde route after infiltration into the interstitium at the basal surface of the tubule. The *zonulae occludentes* are not permeable to the lanthanum tracer. M, mitochondrion. Primary fixation in osmium tetroxide. Section doubly stained with uranyl acetate and lead citrate (× 72,000).

Fig. 9. *Section through apical region of cortical collecting duct from a non-expanded rat showing a junctional complex between two contiguous cells.* Lanthanum injected into the tubule lumen does not penetrate the *zonula occludentes* (arrows) from the luminal surface. TL, tubule lumen; LIS, lateral intercellular space. Primary fixation in glutaraldehyde-formaldehyde. Section doubly stained with uranyl acetate and lead citrate (× 96,000).

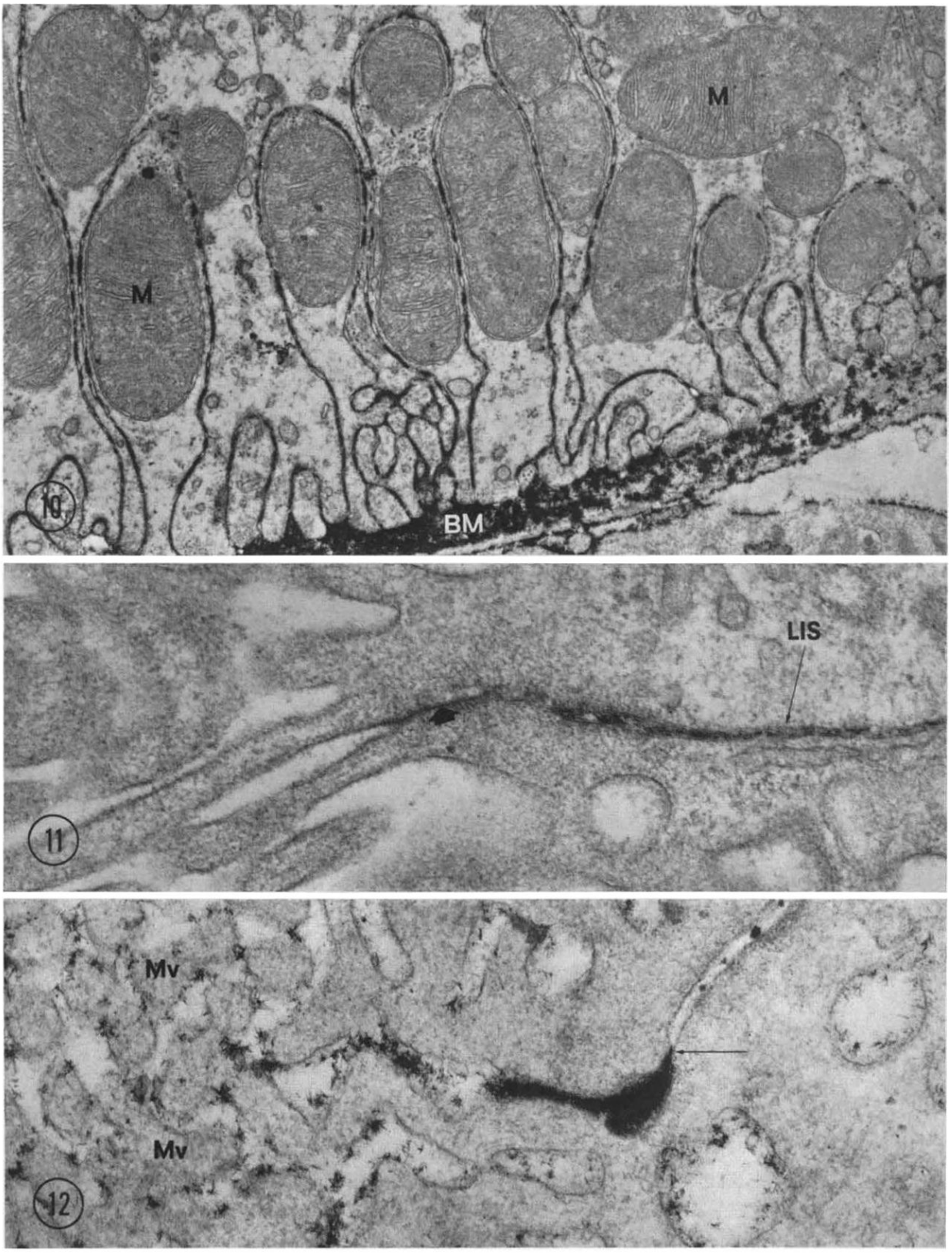

Fig. 10. *Basal region of proximal convoluted tubule from a volume-expanded rat.* Lanthanum infiltrated directly into the interstitium has crossed the basement membrane (BM) and entered the lateral and basilar intercellular spaces. M, mitochondrion. Primary fixation in osmium tetroxide. Section stained with uranyl acetate and lead citrate (× 23,000).

Fig. 11. *Section through apical region of epithelium of proximal convoluted tubule from a volume-expanded rat.* Lanthanum has penetrated the surface after infiltration into the surrounding interstitium. Lanthanum is evident in the lateral intercellular space (LIS). Primary fixation with osmium tetroxide. Section stained with uranyl acetate and lead citrate (× 72,000).

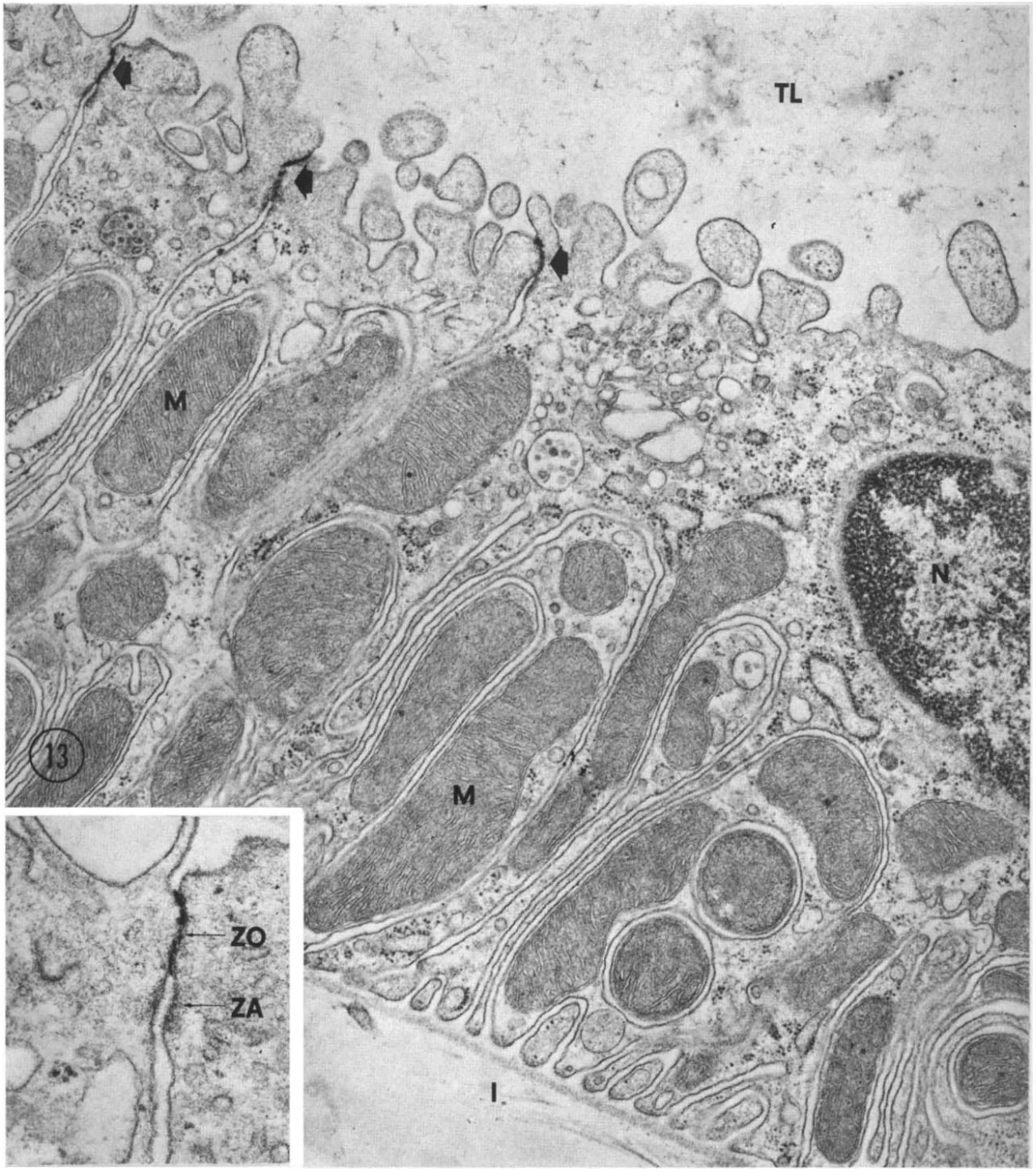

Fig. 13. *Section through the epithelium of a distal convoluted tubule of a volume-expanded rat showing lanthanum penetration of three junctional complexes* (arrows). This tubule was exposed initially to 1 mM $LaCl_3$ before fixation by microperfusion. The insert (lower left) confirms the complete penetration of the *zonula occludens* (ZO) by the lanthanum. ZA, *zonula adherens*; TL, tubule lumen; M, mitochondrion; N, nucleus; I, interstitium. Primary fixation with glutaraldehyde-formaldehyde. Section stained with uranyl acetate and lead citrate. Fig. 13, × 22,500; insert, × 60,000.

Fig. 12. *Section through apical region of epithelium of proximal convoluted tubule from a non-expanded rat.* Oblique and cross sections of microvilli (Mv) are evident on the left. Lanthanum has penetrated the *zonula occludens* (arrow) and small accumulations of lanthanum are present within the region of the *zonula adherens* and lateral intercellular space above the arrow. Tissue preserved by drip-fixation *in situ* with glutaraldehyde-formaldehyde. Section stained with uranyl acetate and lead citrate (× 72,000).

The results of the experiments in which individual renal tubules were initially exposed to 1 mM lanthanum chloride before fixation are shown in Fig. 13. Lanthanum was observed within the tight junctions in both non-expanded and volume-expanded animals. Again, the frequency with which the junctional complexes were found to contain lanthanum deposits appeared to be directly related to the total amount of lanthanum that was present within the lumen of the tubule. No difference was noted in the appearance of the deposits in the two physiological conditions. These findings suggest that the presence of lanthanum within the tight junctions between individual cells of the proximal and distal convoluted tubule can not be explained solely by an alteration in the permeability of the junction in response to the fixation procedure itself.

All results were independent of the type and osmolality of the primary fixative. When the tubules were initially fixed with hypotonic osmium tetroxide and then exposed to lanthanum, the tight junctions also contained lanthanum deposits (Figs. 7, 8 and 11).

In the proximal convoluted tubule measurements were made of the width of the column of lanthanum which penetrated the tight junction or *zonula occludens* in the two physiological conditions. Electron micrographs ranging in magnification from 75,000 to 155,000 were used. Only those junctions were evaluated in which the inner leaflet of the unit membrane and the immediately adjacent clear zone between the inner and outer leaflets of the unit membrane were clearly discernible. In the non-expanded animals the width of the column of lanthanum was 49 Å $\pm$ SEM 3 Å ($N=24$) whereas that in the volume-expanded animals was 48 Å $\pm$ SEM 4 Å ($N=17$; $P=NS$).

Discussion

The results of the present study demonstrate that the tight junction or *zonula occludens* of the proximal and distal convoluted tubule of the rat nephron is permeable to lanthanum, whereas that of the cortical collecting duct appears to be impermeable. If the penetration of lanthanum can be equated with certain functional properties of the tight junction, the findings in the proximal and distal convoluted tubule are compatible with the presence of a high conductance pathway for ion and water movement through a relatively "leaky" tight junction and the lateral intercellular space. The failure of lanthanum to penetrate the tight junction of the cortical collecting duct suggests that such a pathway for ion and water movement is lacking in this segment of the renal tubule, or that it is of considerably less magnitude, or that its nature is such that it exhibits impermeability to lanthanum.

Before relating these morphological findings to known physiological properties of the mammalian nephron and discussing their possible functional significance, the validity of the present morphological techniques requires substantiation. First, the present findings appear to be independent of the effects of fixation. The exposure of individual tubules to La^{3+} as $LaCl_3$ before fixation tends to exclude the possibility that the permeability of the tight junctions of the proximal and distal convoluted tubules was secondary to the fixative. In addition, if the permeability of the tight junctions to lanthanum in the proximal and distal convoluted tubules was the result of a non-specific effect of fixation, it seems likely that the tight junction of the cortical collecting duct should also have been permeable to lanthanum.

The findings also appear to be independent of possible artifactual elevations in intraluminal pressure which might have occurred during microperfusion fixation despite the precautions that were taken. The drip-fixation experiments that were employed to exclude this possibility were prompted, in part, by the results of a recent study of ferritin transport in the flounder tubule. Maunsbach et al [24] observed that ferritin was present in the intercellular spaces within seconds after a solution containing ferritin molecules was intentionally microperfused under high intraluminal pressure into isolated segments of flounder tubule. The findings led to the suggestion that the permeability of the tight junction was transiently increased in response to the increase in intraluminal pressure, thus permitting the ferritin molecules to pass directly through the tight junction into the intercellular space. In the present experiments it is unlikely that the intraluminal pressure was increased when the tubules were preserved by drip-fixation before exposure to the lanthanum tracer. Therefore, it appears reasonable to conclude that the presence of lanthanum in the tight junctions is independent of possible elevations in intraluminal pressure since lanthanum was present within the tight junctions of proximal and distal convoluted tubules preserved by drip-fixation.

The morphological results also correlate well with previous measurements of transepithelial electrical resistance in mammalian proximal and distal convoluted tubules and cortical collecting ducts. In the rat the transtubular electrical resistance in the proximal tubule is 5 to 7 Ω-cm^2 [6]. Recently, Malnic and Giebisch [25] have reported values of approximately 350 Ω-cm^2 for the transepithelial electrical resistance in the distal tubule[1] of the rat. In contrast, the transepithelial electrical resistance in isolated rabbit renal collecting tubules has been found to be 867 Ω-cm^2 [26].

As recently discussed by Frömter and Diamond [2] and Machen, Erlij and Wooding [1], it may be possible to

[1] The distal tubule as defined at the micropuncture table, that is, that segment of the nephron beginning at the *macula densa* and extending to the first junction with another renal tubule, is composed of two morphologically different types of epithelium both of which are accessible to micropuncture. It was not stated explicitly by the authors [25] whether the values for transepithelial electrical resistance were obtained from that region of the distal tubule that morphologically is composed of epithelium characteristic of the distal convoluted tubule (early distal tubule) or the initial collecting tubule or duct (late distal tubule).

divide certain salt-transporting epithelia into two main groups based on certain electrical properties and the ability of lanthanum to penetrate the tight junctions. In one group the tight junction appears to be truly tight, thus resisting penetration by lanthanum. Transepithelial electrical resistance is high and active solute-coupled water fluxes are low. Examples of epithelia that fit these characteristics include the toad urinary bladder and frog skin. The present results, coupled with previous measurements of transepithelial electrical resistance [26], suggest that the mammalian cortical collecting duct may also be included within this group of epithelia. In a second group, the epithelia are characterized by a tight junction that can be penetrated by lanthanum and hence appears to be "leaky." The transepithelial electrical resistance is generally low, while active solute-coupled water fluxes are relatively high. Examples of this type of epithelia for which both morphological and functional data are available include rabbit gall bladder and rabbit ileum [1]. It would appear that the proximal and distal convoluted tubule of the rat kidney can be included in this group of salt-transporting epithelia.

Caution must be exercised in attempting to equate the width of the lanthanum column within the *zonula occludens* to the size of a paracellular channel for ion and water movement. First, our knowledge of the structure of the *zonula occludens* in the proximal convoluted tubule is severely limited. This is largely due to the fact that newer morphological techniques available to evaluate structural configuration of cell junctions such as freeze-fracture and ultrastructural demonstration of responses to proteolytic enzymes and divalent cation-chelators have not been applied to the cell junction of the mammalian proximal convoluted tubule. Secondly, it is quite possible that lanthanum may interact with various structural components within the *zonula occludens* resulting in the formation of an image that does not necessarily reflect the actual size of the channel. The latter possibility is well exemplified in the original characterization of the gap junction in the mouse heart by Revel and Karnovsky [16]. For many years this junction was thought to be of the occludens type. However, tissue stained en bloc with uranyl acetate revealed the presence of an 18 to 20 Å spacing or "gap" between the outer leaflets of the two adjacent plasma membranes in the cell junction. Yet, when the tissue was exposed to lanthanum, a 55 Å electron dense "gap" was present where the 20 Å gap was previously observed. The area occupied by the lanthanum was equal to the width of the original 18 to 20 Å gap observed with en bloc staining plus the width of the outer leaflets of the two apposed cell membranes. Tangential sections through the gap junction revealed the presence of an hexagonal array of subunits formed in part by the outer leaflets of the two adjacent plasma membranes. The hexagonal subunits were separated by a 15 to 20 Å space which was freely permeable to lanthanum. This complex substructure involving the original 20 Å "gap" and the outer leaflets of the two apposing plasma membranes was outlined by the lanthanum tracer and accounted for the 55 Å electron dense spacing. It is interesting to note that in the present study the 48 to 49 Å space occupied by lanthanum in the *zonula occludens* of the proximal convoluted tubule is very close to the 55 Å spacing in the gap junction of the mouse heart, but en bloc staining of this tight junction has consistently failed to reveal a gap or space between the outer leaflets of the two apposing plasma membranes [23, unpublished observations]. However, fixation with $KMnO_4$ or en bloc staining often reveals the presence of a fusion line between the two apposing cell membranes giving rise to a characteristic pentalaminar structure [23]. Despite the absence of the gap or spacing it is possible that the lanthanum may be outlining a more complex substructure, not necessarily hexagonal in type, within the *zonula occludens* of the proximal convoluted tubule. If so this could explain the 46 to 48 Å spacing demonstrated by the lanthanum. In the mouse heart the hexagonal array of subunits in the gap junction was evident through the use of thin sections and lanthanum tracer. Thus far, we have not observed such a configuration in the tight junction of the rat proximal convoluted tubule. At present we simply choose to stress the fact that the *zonula occludens* of the proximal and distal convoluted tubule is permeable to lanthanum and the corresponding region in the cortical collecting duct is not under the conditions of the present experiment. The functional and structural significance of the width of the lanthanum column in the proximal convoluted tubule requires further study.

The failure to demonstrate a difference in the size or the general appearance of the lanthanum column penetrating the *zonula occludens* in the proximal convoluted tubules of volume-expanded versus non-expanded animals could be due to a variety of reasons. First, demonstration of widening of the leaky tight junction during volume expansion presupposes that increased backflux of ions and water does occur. Although considerable indirect evidence can be marshalled in support of the existence of a functional paracellular shunt pathway for ion and water movement, it is also possible that decreases in net sodium and water absorption in the proximal tubule during volume expansion could be due to the presence of humoral factors or to an increase in hydrostatic pressure, both of which are known to decrease net sodium transport [27]. If it is assumed that a functional paracellular shunt does exist in the proximal convoluted tubule and increased backflux of sodium and water contributes to decreased net reabsorption as suggested by the studies of Boulpaep [28] in *Necturus* proximal tubule during saline expansion and Bank, Yarger and Aynedjian [29] in the proximal tubule of the rat during renal venous constriction, the change in size of a potential channel may be of the order of only a few angstroms and thus so small as to be undetectable with the morphological techniques utilized in the present study. Alternatively, a complex macromolecular substructure may be present within the region of the *zonula occludens* which can alter internal

resistance of the junction to ion and water flow without affecting the appearance of the junction as visualized by lanthanum staining. It is also possible that an increase in the number of channels, rather than an increase in the width of a channel(s) can occur with increased backflux. However, this explanation is less attractive for at least two reasons: first, the frequency with which tight junctions were observed to contain lanthanum was the same in the two conditions that were studied; second, it is probably more accurate to visualize the potential channel through the tight junction as a continuous space which circumscribes the cell rather than a group of individual channels.

Acknowledgments

Supported in part by grants AM 13845 and HL 5848 from the U.S. Public Health Service. Dr. Tisher is the recipient of Research Career Development Award HE 440074 from the U. S. Public Health Service; Dr. Yarger is an Rampersand & E Associate of the Veterans Administration. The authors express their appreciation to Mrs. Helen Parks, Mrs. Kathy Blake, and Mrs. Betty Waller for technical assistance and to Mrs. Jessie Calder for preparation of the illustrative material.

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